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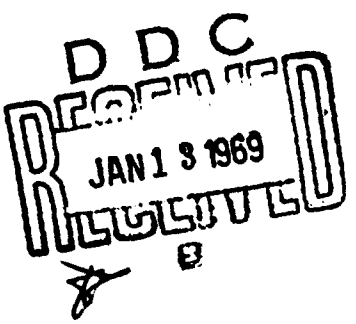

DEPARTMENT OF THE ARMY
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ECOLOGICAL FEATURES OF SPORE FORMATION IN THE FUNGUS Peronospora tabacina Adam

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Oospores, the winter form of the fungus Peronospora tabacina A. which is the causative agent of peronosporosis in tobacco, are formed in lesions of tissues of the tabacco plant, primarily in the leaves. However, in raw tobacco from the harvests of 1960, 1961 and 1962 from different oblasts in the Ukraine (Transcarpathia, Ternopol', Chernogov, Kiev and the Crimea) the oospores were very rarely found. Under conditions prevailing in 1961 and 1962 they were formed more frequently and more thickly in Belorussia.

A comparison of results from tobacco analysis and meteorological data from the period of peronosporosis development showed that the dominant factor in oospore formation is the humidity of the atmosphere [1, 2] .

In order to make a detailed study of the effect of environmental conditions on oospore formation in P.tabacina special field studies were carried out in 1963 in Ternopol and Transcarpathia Oblasts.

Observations were made of peronosporosis spots from the appearance of the chlorotic spot until its disappearance. The observation periods were marked by different meteorological conditions. As we can see from Table 1 the oospores were observed in spots formed on leaves during the period from August 14 to 26 (Ternopol' Oblast). Then there were lasting rains almost every day which produced high atmospheric humidity (average 80% and in the leaf zone 87 - 90%). In many cases the damaged tissue was wet and looked like wet spots.

In the last periods characterized by very dry weather there was no spore formation. Under such conditions in 1962 oospores also did not form [2] .

TABLE 1

METEOROLOGICAL CONDITIONS DURING SPORE FORMATION OBSERVATION

Dates	time (days)	av.temp (°)	av.humidity (%)	total precip. (mm)	days with rain	type of rain	oospores
			TERNOPOL' OBLAST				
20-27 July	7	24.3	65	1.4	1	brief	none seen
29 Jul-3 Aug	9	23.0	69	6.2	1	"	"
14 - 26 Aug	13	19.2	80	56.3	9	continuing fog	spores formed
1 - 10 Sept	10	20.4	73	13.0	1	brief heavy	none seen
			TRANSCARPATHIA				
20 - 29 July	9	24.7	62	1.6	1	brief	none seen
31 Jul - 9 Aug	9	26.1	60	0			" "
5 - 15 Aug	10	22.9	71	21.2	1	continuing	

Thus our observations showed that spore formation requires high atmospheric humidity and prolonged wetness of leaves and this is provided by frequent lasting rain and fog. As for the effect of temperature on oospore formation, our observations did not lend any significance to this factor. In the literature there are indications that it is temperature and not humidity that plays the decisive role in spore formation; extensive oospore formation can be observed at a temperature of 16-27 and a relative humidity of 50 - 60% [3].

Our observations in 1962 [1] and 1963 did not confirm these data. At a temperature of 16 - 20 and a relative humidity of 60 - 76% there was no oospore formation but during a period with the same temperature: 19 and 20° oospores were formed if the humidity was higher (Cf. Table 1).

There is no basis for rejecting the importance of temperature in oospore formati/n. This problem must be studied experimentally. We believe that temperature may have some importance but humidity is the decisive factor.

Our conclusions as to the role of humidity is also confirmed by the results of experiments made in greenhouses. We were observing plants in pots. The plants which had been hand-inoculated with conidia of P.tabacina were severely affected. They remained affected to the very end of the observation under conditions of very high humidity (average 90 - 92% with fluctuations to 84 - 99%). On the 8th - 10th day after the disease started 73% of the leaves showed oospores distributed in spots at many sites on the leave surface.

Oospores were also observed on plants in hot beds when planted close together with immoderate watering, i.e. with high humidity prevailing. They were also found in brown tissue and in green tissue of the affected leaves. The detection of oospores in green tissue gave reason for believing

that they develop in leaves from the very beginning of the disease. An examination of the research material confirmed this. For instance a micro-analysis of affected green tissue showed a large number of oogonia and oospores. In some oogonia the contents looked granular and uniformly distributed throughout the body, in others the process of oospore formation was beginning.

The oospores were in different stages which makes it possible to divide them into four groups:

I. Oospores pinkish-white or pinkish-yellow in color with large-grained contents and no internal membrane but the outer membrane adhered closely to the walls of the ooginium.

II. Oospores of same color and structure but internal membrane already formed and outer separated from the oogonial wall.

III. Oospores yellow or reddish-yellow in color. Contents finely granular. Inner and outer membranes clearly differentiated.

IV. Oospores normally colored red or golden brown. Contents homogeneous, occasionally with remnants of fine granularity.

Thus even in the green tissue the studied stages in the oospore development are present and this indicates that the formation process takes place at an early stage in the development of the disease, even before the damaged tissue has died away. At the same time there are indications in foreing literature that oospores develop even in the dead tissue [4]. Our data refute such statements. We observed what happened to imperfectly developed oospores (groups I and II) by the following study: immediately after tobacco leaves were broken we selected bits of damaged tissue - green and reddened - with oospores. Some bits were soaked in water and placed in a moist chamber, others were kept at room temperature in a dry state.

It was found that in green tissue under wet conditions the process of oospore formation continued while there was no further oospore development in the same tissue when it was kept dry. The oospores with imperfect development died. They took on a dark red appearance and their contents were not visible. After the oospores were processed with Javelle water which produced dissolution of the outer membrane [3], it was found that the inner membrane had not formed in them and the central portion was completely granular.

This study showed that for normal development the oospores require moistness of the leaves from the very onset of the disease.

An analysis of the reddened tissue showed mostly formed oospores but some were of imperfect development. After this tissue was kept for 10 days under humid conditions there was no further development of the unformed oospores was seen. So oospores which had not finished development in the leaves before the damaged tissue died remained unformed and died.

Our research gives us reason to believe that in practice oospores may continue their development in tobacco leaves even after the leaves have been picked if there are chlorotic or slightly reddened peronosporosis spots on the leaves at the time; if there is prolonged wet weather before the leaves are broken off or even when the damaged leaves are shade-dried under wet conditions with weak ventilation, particularly in preliminary "tomlins". The dead peronosporosis spots did not create a threat for further oospore development even under damp weather conditions.

Oospores do not always develop even under favorable weather conditions. In our field studies oospores were found on an average in less than 30% of the analyzed spots.

A detailed study of our research material showed that oospore development depends not only on weather conditions but other environmental factors which favor or restrict development. An evaluation of the effect of these factors is given for meteorological conditions of August 14 - 26 (Table 1), i.e. the period when oospore formation was observed under field conditions. The results are given in Table 2.

It was found that plant growing conditions have an effect on oospore formation. For instance, oospores develop frequently when plants are close growing but among wider spaced plants there were none.

Oospores developed on leaves of the lower rank. The same thing was observed in 1962 [3]. Oospore development in the lower rank, predominantly in closely planted beds, is caused by the accumulation of moisture in the growing zone of the leaves under study; in the lower rank of close-growing plants the average relative humidity was 90%, normal 87%, widely spaced 80 - 82% (optimal for oospore development - 90%).

Our observations showed that oospores are found on leaves with certain damage signs, i.e. of a diffuse type: damaged tissue of a reddish, greenish or greenish-red color with indistinct edges, including a large part of the leaf surface, not infrequently the entire leaf, covered with a conidial bloom of P. tabacina on the lower side and sometimes on the upper as well. On the root the damaged tissue has the appearance of wet spots and turgor is reduced. This type of affection is observed predominantly on lower leaves in dense plantings. On middle and upper leaves peronosporosis spots are most frequently observed with have distinct edges with healthy tissue. Oospores do not develop in such spots.

Leaves with the diffuse type of infection were also found in plants set out late in the field. These plants were slower in growth and development and were covered by normally developed plants. Being thus in the shade, particularly in dense plantings, they had low young tissue, were badly infected with peronosporosis with identical infection on leaves of all levels. A microanalysis of the affected tissue showed masses of oospores not only on lower but upper leaves.

EFFECT OF VARIOUS FACTORS ON OOSPORE FORMATION Table 2
(Peronospora tabacina)

spacing (in cm)	var. Gostrolist B2747: spots analyzed on leaves	no. spots w/oospores total	low leaves	middle leaves	high	var. Sobol'chs'kyy: spots analyzed on leaves	no. spots w/oospores total	low leaves	middle leaves	high
40 x 20	60	43	43	0	0	40	14	14	0	0
60 x 20	40	18	18	0	0	30	0	0	0	0
60 x 40	50	0	0	0	0	30	0	0	0	0
total	150	61	61	0	0	100	14	14	0	0

Thus it has been determined that the condition of the plants is of important for the process of oospore formation; the lower young tissue is not only more susceptibe to infection with peronosporosis but in it the fungus is capable of developing the winter stage to a greater degree.

The relation between the degree of plant susceptibility and oospore development was brought out as early as 1961. It was found that under identical conditions oospores did not develop on the moderately affected Sobolchs'kyyy variety while oogonia and oospores were detected on Nicotinia glutinosa which is susceptible to peronosporosis [1].

The importance of immunological features of the variety with regard to the process of oospore formation was also discovered in 1963 by making a comparative study of oospore formation on two tobacco varieties: the highly susceptible Gostrclist B2747 and the moderately susceptible Sobol'chs'kyy. Under conditions of humid weather a large number of oospores could be found on Gostrolist but on Sobol'chs'kyy they occurred only in small numbers in certain spots in dense plantings (Table 2). Also on plants which had delayed development the oospores formed much more frequently on Gostrolist than on Sobol'chs'kyy. In all cases where oospores were found the leaves had the characteristic diffuse type of infection.

Under favorable meteorological conditions oospores may be formed on plants grown in different feeding conditions. We observed oospore formation on plots fertilized with NPK and PK and on plots without fertilizer. Oospores were formed most frequently in situations where leaves most often showed the diffuse type of infection. For instance where there was a lot of manure used (40 tons/hectare) and the tobacco was greatly affected by peronosporosis, these leaves occurred rather frequently and there was massive oospore formation.

Thus our research and observations have shown that the more susceptible a plant is to peronosporosis, the more frequently we find leaves with a diffuse type of infection and the earlier oospore formation occurs.

CONCLUSIONS

1. Oospore formation of P.tabacina is affected by a number of factors: meteorological, biological and agronomic. Decisive among them are weather conditions; biological and agronomic factors may favor or hinder oospore formation.

2. The oospore formation process starts with the green tissue in the affected tobacco leaf, i.e. from the very start of the disease. In the green tissue one may also find oogonia as well as the later stages of oospore development, including oospore formation.

3. Oospore formation requires high atmospheric humidity (prolonged rain and fog), starting from the appearance of chlorotic spots on the leaves and lasting until they die.

4. With favorable conditions in the field oospores develop largely in closely spaced tobacco plantings on lower leaves of normally developed plants. In plants which have been set out late, lagging in growth and development, oospores form quite often and in large numbers on leaves of any level on the plant.

5. Oospore formation depends on immunological properties of plants. They are formed most frequently and earlier on plants which are more susceptible to infection.

6. A high level of fertilization (40 tons/hectare) is favorable to oospore formation.

7. Oospores also develop in hot beds where plants are close together and overwatered and in greenhouses where there is high humidity (90%).

8. Oospores are found in leaves with diffuse type of infection.

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